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ABSTRACT

The safety of the first generation oncolytic HSV has been well documented. Retaining this safety profile is mandatory for potential clinical applications of the fusogenic oncolytic HSV. The fusogenic oncolytic HSV has thus been uniquely designed and constructed as such that the syncytia formation from the virus requires viral DNA replication in the targeted cells. As oncolytic HSV can only initiate viral replication in tumor cells, this restricts the syncytial formation from virus infection to malignant cells only. Therefore fusogenic oncolytic HSV should be no more toxic than its parental construct. Nonetheless, we proposed in the year 2 of this funded project to conduct extensive studies in animal models to confirm its safety in vivo. The results obtained so far from these experiments have demonstrated that the fusogenic oncolytic HSV is indeed not significantly more toxic than the nonfusogenic virus, confirming our original hypothesis that the fusogenic virus is safe for potential clinical application.

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INTRODUCTION

The safety of the first generation oncolytic HSV has been well documented (1-3). Retaining this safety profile is mandatory for potential clinical applications of the fusogenic oncolytic HSV. Studies conducted during the first year of this funded project have demonstrated that the fusogenic oncolytic HSV has a dramatically enhanced antitumor tumor effect against both orthotopic and metastatic prostate cancer (4). We believe that due to its unique design and construction (syncytia formation from the virus requires initiation of viral DNA replication and oncolytic HSV can only initiate replication in tumor cells), fusogenic oncolytic HSVs such as Synco-2 will be no more toxic than its first generation counterpart, a minimal requirement for future use in patients. In the original Statement of Work, we proposed to spend year 2 of the project period to conduct experiments to extensively evaluate the toxicity of the fusogenic oncolytic HSV in animal models.

BODY

We conducted experiments to directly compare the toxicity of the fusogenic and non-fusogenic oncolytic HSVs by injecting the viruses systemically into immune-competent mice at escalating doses.

Experimental design and procedure. Six-week-old immune competent BALB/c mice were injected through the tail vein with either the fusogenic Synco-2 or Baco-1 at a low and high doses: $5X10^7$ or $3X10^8$ pfu (the dose of $3x10^8$ in 200 µl of solution is the maximum that could be injected systemically into mice, given the modest scale of virus preparation). Mice in negative control group were given the same volume of PBS.

Incidence of mortality. For evaluation of mortality after oncolytic HSV administration, animals were observed for a six-month period. Shortly after virus injection, two mice receiving the higher dose of Baco-1 became inactive, but they recovered in less than 3 h. Otherwise, there were no animal deaths

or evidence of disease during the observation period in either groups (Table 1).

Table 1. Mortality after systemic administration of high doses of oncolytic HSVs

Viruses	titer	Route admin.	No. mice	mortality
Baco-1	$5X10^7$	i.v.	5	0
Baco-1	$3X10^8$	i.v.	5	0
Synco-2	$5X10^7$	i.v.	5	0
Synco-2	$3X10^8$	i.v.	5	0
PBS	200 μl	i.v.	5	0

Histopathological findings after systemic administration of oncolytic HSVs

For determining histopathological abnormalities, mice were killed 1 week after virus administration. Following euthanasia, a complete necropsy was performed. Tissue samples were obtained from major organs including brain, heart, intestines, kidney, liver, lung, pancreas, spleen, and stomach. About one-third of the freshly obtained organ tissues were immediately frozen for determining virus distribution (details in the next section). Tissue sections were prepared from the remaining organ mass by the College's pathology core facilities and then examined for signs of histopathological changes. Only mild histological abnormality was occasionally seen in liver sections from mice receiving administration of either Baco-1 or Synco-2 (scored as either + or ±). There was no major difference on the severity of this abnormality among the fusogenic and nonfusogenic oncolytic HSVs. However, there was a slight difference on the severity of the liver pathological abnormality in animals receiving low and high doses of Synco-2. No obvious pathological changes were identified in any other organs from mice in all the three treatment groups (Table 2). During the microscopic examinations, particular attention was made to look for syncytial formation from tissue sections

prepared from mice received Synco-2 administration. No syncytial formation was visible, even in liver sections where mild tissue abnormality was noticed.

Table 2. Histopathological scores after systemic administration of oncolytic HSVs

organs	PBS	Baco-1		Synco-2	
	200μl	$5X10^7$	$3X10^8$	$5X10^7$	$3X10^8$
Brain	—	—	—	—	—
Heart	—	—	—	—	—
Intestine	—	—	—	—	—
Kidney	—	—	—	—	—
Liver	—	÷	÷	±	÷
Lung	—	—	—	—	—
Pancreas	—	—	—	—	—
Spleen	—	—	—	—	—
Stomach	—	—	—	—	—

Quantification of retrievable virus from the major organs

One third of the fresh organ tissue samples from Table 2 was used for virus isolation. The tissue slices were quick-frozen. Tissue homogenates were prepared in Dulbecco's modified essential medium (DMEM), using Dunce homogenizer. After centrifugation at 700 X *g* at 4^0C for 10 min, the supernatants were serially diluted in DMEM and virus titer titrated on Vero cells. The results were shown in Table 3. Small quantities of oncolytic HSVs were retrievable from livers at the time when tissue samples were taken. Residual virus was not detected in other organs except in the lung, where a low level of Baco-1 was detected in animals receiving the high dose of Baco-1 administration.

Table 3. Live virus distribution in major organs after systemic administration of oncolytic HSVs

organs	PBS	Baco-1		Synco-2	
	200μl	5X10^7	3X10^8	5X10^7	3X10^8
Brain	ND*	<10pfu/ml#	<10pfu/ml	<10pfu/ml	<10pfu/ml
Heart	ND	<10pfu/ml	<10pfu/ml	<10pfu/ml	<10pfu/ml
Intestine	ND	<10pfu/ml	<10pfu/ml	<10pfu/ml	<10pfu/ml
Kidney	ND	<10pfu/ml	<10pfu/ml	<10pfu/ml	<10pfu/ml
Liver	ND	<10pfu/ml	20±3pfu/ml	<10pfu/ml	15±2pfu/ml
Lung	ND	<10pfu/ml	10±2pfu/ml	<10pfu/ml	<10pfu/ml
Pancreas	ND	<10pfu/ml	<10pfu/ml	<10pfu/ml	<10pfu/ml
Spleen	ND	<10pfu/ml	<10pfu/ml	<10pfu/ml	<10pfu/ml
Stomach	ND	<10pfu/ml	<10pfu/ml	<10pfu/ml	<10pfu/ml

*Not detectable; #The least diluted samples was 1:5 (i.e., adding 100 µl of the homogenized tissue solution to 400 µl of DMEM to make a total of 500 µl for infection of Vero cell monolayer seeded in 6-well plates. So if no virus was detected, then it means that the amount of the virus in the organ was <10 pfu/ml.

KEY RESEARCH ACCOMPLISHMENTS

The results obtained from the extensive in vivo toxicity studies demonstrated that

- Systemic administration of the fusogenic Synco-2 in a relative high dose did not cause any animal death.
- Systemic administration of oncolytic HSVs caused a mild histopathological abnormity. However, there was no significant difference on the severity of liver toxicity between the

fusogenic and non-fusogenic oncolytic HSVs.

- At one week after systemic administration of the oncolytic HSVs, residual viruses were not detectable in the majority of organs. Only low level of residual virus was detected in liver and lung (only after high dose administration of Baco-1). There was no significant difference between the amount of virus retrieved from mice receiving either Baco-1 or Synco-2.

REPORTABLE OUTCOMES

1. Conference presentation: Dr. Zhang was one of three invited overview speakers at the 29th International Herpesvirus Workshop (held at Reno, Nevada, July 25-30, 2004). Title of talk: HSV vectors for gene therapy of solid tumors and genetic diseases.

2. Invited corporate seminar presentation: Dr. Zhang was invited by Immusol, Inc (San Diego, USA) to deliver a seminar, on September 23, 2004. Title of talk: Oncolytic virotherapy for solid tumors including prostate and ovarian cancer.

CONCLUSIONS

The extensive in vivo toxicity comparison of the fusogenic Synco-2 and the non-fusogenic oncolytic Baco-1 demonstrate that the former is not significantly more toxic that the later, whose safety profile has been well documented. These results confirm our original hypothesis that due to the unique design of Synco-2, it posses higher therapeutic efficacy without significantly increasing its in vivo toxicity.

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APPENDICES:

None